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Genomic Rearrangements Associated with Antigenic Variation in *Campylobacter coli*

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***Campylobacter coli* and *Campylobacter jejuni* share a limited number of highly conserved DNA sequences with members of the family *Enterobacteriaceae*. One of these sequences was cloned from *C. coli* VC167, and the region of homology to the enteric sequences was determined to be confined to a 700-base-pair region. The DNA represented in this clone undergoes a programmed, reversible rearrangement in VC167 that is associated with flagellar antigenic variation.**

The genus *Campylobacter*, which includes a number of important human and animal pathogens, comprises a group of spiral, gram-negative microaerophilic organisms of uncertain taxonomic status. Recent analyses of 16S rRNA sequences of campylobacters indicate that the genus forms a previously undescribed basic eubacterial group (10). *C. jejuni* and *C. coli* are the major human pathogens and are frequent causes of bacterial enteritis, particularly in children. Despite their importance as human pathogens, very little is understood about the pathogenesis of *Campylobacter* spp., due in large part to the absence of a system of experimental genetics. There have been several reports on the instability of certain traits in *Campylobacter* spp. Colonial variation has been observed in *Campylobacter* spp. (13–15), and there have been reports of virulence enhancement by passage through mice (7) or rabbits (9). Caldwell et al. (3) have observed that *C. jejuni* can undergo a bidirectional transition or phase variation between flagellated and nonflagellated forms in vitro and that passage through a rabbit intestine markedly favors the flagellated phenotype. Harris et al. (5) have shown that some strains of *C. coli* and *C. jejuni* alternately express distinct flagellin molecules of different antigenic specificities (antigenic variation). The present study demonstrates that a strain of *C. coli*, VC167, undergoes a DNA rearrangement concomitant with flagellar antigenic variation. This rearrangement could be detected by using a probe containing a defined DNA sequence that *Campylobacter* spp. share with members of the family *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *C. coli* VC167, serogroup LIO8, is a clinical isolate from the United Kingdom. *C. jejuni* HC, of unknown LIO serotype, is a clinical isolate from the United States. Strains of the *Enterobacteriaceae* were obtained from the Walter Reed Army Institute of Research collection: *Salmonella typhi* 643, *Salmonella typhimurium* LT2, *Shigella flexneri* 2457-O, and *Escherichia coli* HB101. The enterobacteria were cultivated in Luria broth (8); campylobacters were grown routinely on sheep blood agar plates at 37°C in an atmosphere of 85% nitrogen, 10% carbon dioxide, and 5% oxygen.

Selection of cells producing flagella of different antigenic specificity. Selection of VC167 cells producing antigenic phase 1 or phase 2 flagella was as described by Harris et al. (5).

DNA extractions. Total bacterial DNAs were isolated by the method of Hull et al. (6). For initial screening of recombinant DNA clones, plasmid DNAs were prepared by the method of Birnboim and Doly (1); large-scale plasmid preparations were obtained by the method of Clewell and Helinski (4) followed by banding in cesium chloride-ethidium bromide gradients.

Southern blot analyses. DNAs (generally 2 to 3 μg per strain) were digested to exhaustion with 20 to 100 U of restriction endonuclease as recommended by the supplier (New England BioLabs, Inc., Beverly, Mass.) and electrophoresed on agarose slab gels. Transfer to nylon membranes (GeneScreen Plus; Du Pont Co., Wilmington, Del.) was done as recommended by the supplier. Hybridizations were done for 18 h at 60°C in 4× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate)–5× Denhardt solution (8)–1.0% sodium dodecyl sulfate–100 μg of calf thymus DNA per ml–5×10^6 dpm of probe per ml. Four washes of 30 min each were performed in 0.5× SSC at 60°C. DNAs were radiolabeled by nick translation by using [α-^{32}P]dCTP (Du Pont-New England Nuclear Corp., Wilmington, Del.) as described by Maniatis et al. (8).

Molecular cloning. DNA from *C. coli* VC167 was digested with *Hin*dIII restriction endonuclease and separated on a sucrose density gradient as described by Maniatis et al. (8). The gradients were fractionated, and selected fractions were electrophoresed on a 0.7% agarose slab gel. The DNA was transferred to a nylon membrane and probed with nick-translated ^{32}P-labeled total DNA from *S. typhimurium* LT2. Fractions containing the most intense hybridization signals were pooled, dialyzed, and ligated into pBR322 that had been cleaved with *Hin*dIII and dephosphorylated with bacterial alkaline phosphatase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The ligation mixture was transformed into competent *E. coli* DH5 cells (Bethesda Research Laboratories). Clones were selected on Luria agar plates containing 50 μg of ampicillin per ml. Plasmid DNAs from individual ampicillin-resistant, tetracycline-sensitive clones were prepared by the method of Birnboim and Doly (1), digested with *Hin*dIII, and analyzed by Southern blot hybridizations, with *S. typhimurium* LT2 DNA as the probe.

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RESULTS

Homology between *Campylobacter* spp. and members of the *Enterobacteriaceae*. *Campylobacter* species are taxonomically remote from members of the *Enterobacteriaceae* and would not be expected to share significant nucleotide sequence similarities. Nevertheless, when total chromosomal DNA from *C. jejuni* HC was nick translated with [^{32}P]dCTP and used to probe *Hin*dIII-digested DNA from various members of the *Enterobacteriaceae* by Southern blot analysis, a number of bands of varying intensities were observed, ranging in size from approximately 2 kilobases (kb) to >20 kb (Fig. 1). When total DNA from *S. typhimurium* LT2 was nick translated and used to probe *C. coli* or *C. jejuni* DNA, discrete bands were also observed, but the total number was considerably fewer; the sizes ranged from approximately 4.6 to 5.2 kb when *Campylobacter* DNAs were digested with *Hin*dIII endonuclease and from 3.0 to 9.4 kb when they were digested with *Eco*RV endonuclease (see Fig. 4). When total *S. flexneri* or *E. coli* DNAs were used to probe *Campylobacter* DNAs, the same patterns were observed, indicating that these bands most likely represent a set of sequences held in common among the different organisms (data not shown). The stringency conditions of the washes used for these blots were equivalent to a T_m − 23.7°C for the enteric DNAs (51% G+C) and a T_m − 15.5°C for *Campylobacter* DNAs (31% G+C). When blots similar to that shown in Fig. 1 were washed under increasing conditions of stringency, bands of decreased signal were still visible under wash conditions equivalent to T_m − 12°C for 51% G+C and T_m − 3.9°C for 31% G+C. This thermal stability of the hybridized duplexes indicates that the observed homology is relatively exact (8).

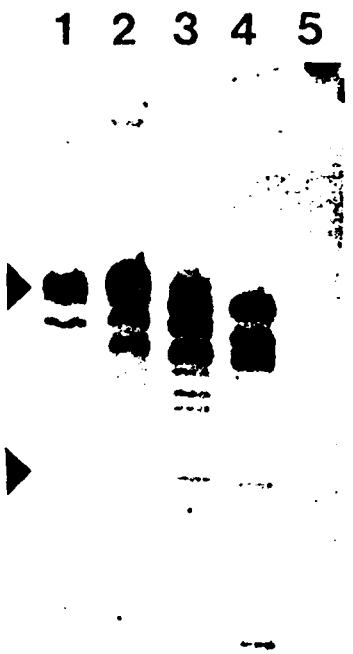

FIG. 1. Autoradiogram of Southern blot of *Hin*dIII-digested total DNA from different members of the *Enterobacteriaceae* probed with ^{32}P-labeled *C. jejuni* HC DNA. Lane 1, *S. typhi* 643; lane 2, *S. typhimurium* LT2; lane 3, *S. flexneri* 2457-O; lane 4, *E. coli* HB101; lane 5, bacteriophage lambda. The arrowheads indicate the approximate positions of size standards: top, 23.1 kb; bottom, 4.3 kb.

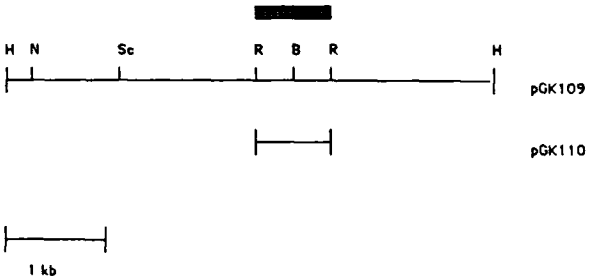

FIG. 2. Physical map of *C. coli* VC167 DNA cloned in pGK109 (the vector portion of the plasmid is not shown). The hatched area indicates the region that hybridizes to *S. typhimurium* LT2; this corresponds to the DNA subcloned into pBR322 to generate pGK110. Restriction sites: H, *Hin*dIII; B, *Bam*HI; S, *Sal*I; Sc, *Sca*I; R, *Rsa*I; N, *Nde*I.

A piece of DNA containing one of the homologous sequences was cloned from *C. coli* VC167. Plasmid pGK109 was identified that contained a 5.2-kb insert of VC167 DNA that strongly hybridized with a probe of total *S. typhimurium* LT2 DNA. When pGK109 was nick translated and used to probe DNA from representative *Enterobacteriaceae* strains, multiple bands similar to those shown in Fig. 1 were observed (data not shown). These data indicate that pGK109 contains a *C. coli* sequence that is present in multiple copies in the genome of several enteric strains. A partial restriction map of the insert is shown in Fig. 2. The region of homology between pGK109 and the enteric bacteria was determined by subcloning and restriction enzyme analysis to be centered around a 700-base-pair *Rsa*I fragment, although the exact endpoints of homology remain to be determined. This 700-base-pair *Rsa*I fragment was subcloned into the *Eco*RV site of pBR322 to generate plasmid pGK110 and used to probe DNA from members of the *Enterobacteriaceae*. The results (Fig. 3) show the same multiple banding pattern. The intensely hybridizing bands observed with *S. flexneri* (lane c) were the only bands observed when pBR322 was used to probe identical blots and probably represent the cryptic ColE1-like plasmids reported to be present in *S. flexneri* (11).

Genomic rearrangements of the sequence during phase variation in strain VC167. The limited number of highly conserved DNA sequences shared by such diverse organisms as the enteric bacteria and *Campylobacter* spp. could represent rRNA genes, known to be highly conserved in eubacteria, or insertion sequences, whose promiscuous transmission among different bacterial genera can be mediated by plasmids. Harris et al. (5) have described antigenic variation in the flagellin molecule of *C. coli* VC167. Phase variants can be selected by growth of the organism in antiserum prepared against one particular antigenic phase of flagella. To determine whether the sequence cloned in pGK109 was involved in any DNA rearrangements associated with these surface antigenic changes, we did the following experiment. DNA was prepared from a culture of VC167 expressing phase 1 flagella. The strain was grown in the

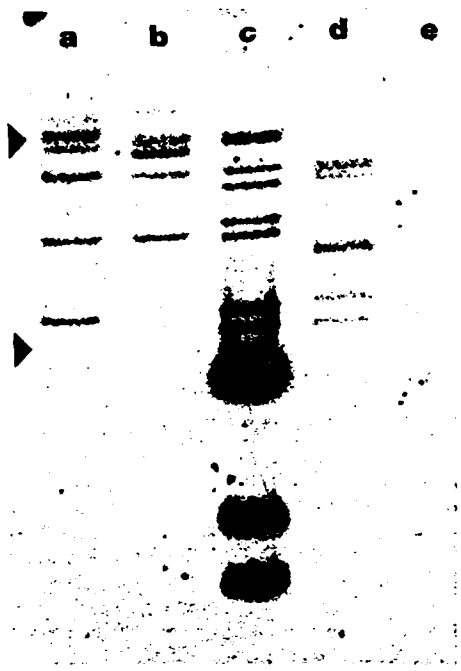

FIG. 3. Autoradiogram of Southern blot of *Hin*dIII-digested DNAs probed with pGK110. Lane a, *S. typhimurium* LT2; lane b, *S. typhi* 643; lane c, *S. flexneri* 2457-O; lane d, *E. coli* HB101; lane e, bacteriophage lambda. The arrowheads indicate the approximate positions of size standards: top, 23.1 kb; bottom, 4.3 kb.

presence of anti-phase 1 serum, phase 2 cells were purified as described by Harris et al. (5), and DNA was extracted from them. Phase 2 cells were grown in the presence of anti-phase 2 serum, phase 1 cells were reisolated, and DNA was extracted from them. The DNAs were restricted with *Eco*RV endonuclease and probed with *S. typhimurium* LT2 DNA. The results (Fig. 4A) indicate that a reversible rearrangement occurred. A 6.6-kb band remained constant in all three strains; however, *S. typhimurium* LT2 DNA hybridized to a 9.4-kb band from phase 1 cells and an 8.4-kb band from phase 2 cells. The DNAs of two independently isolated sets of antigenic variants of VC167 were compared, and they showed the same rearrangements. The results obtained when VC167 phase 1 or phase 2 DNA digested with two restriction enzymes was probed with pGK109 DNA are shown in Fig. 4B; the rearrangement was again observed after digestion of the two DNAs with *Eco*RV but not when the DNAs were digested with *Hin*dIII. Since the *Hin*dIII fragment of VC167 DNA cloned in pGK109 does not contain any *Eco*RV sites, this indicates that the endpoints of the observed chromosomal DNA rearrangement lie within an *Eco*RV fragment which includes the *Hin*dIII fragment cloned in pGK109. Patterns identical to that shown in Fig. 4B were seen when the pGK110 subclone of pGK109 was used as the probe, further suggesting that the DNA rearrangement probably occurs at a site on the VC167 chromosome adjacent to, but not within, the cloned *Hin*dIII fragment.

DISCUSSION

We showed that *C. coli* VC167 undergoes a reversible genomic rearrangement associated with flagellar phase variation. Rearrangements controlling expression of surface antigens are documented for numerous bacterial and parasitic pathogens (2). Although we did not establish a causal relationship between the rearrangement and the antigenic variation in VC167, the two phenomena occurred concomitantly in two independently isolated sets of VC167 antigenic variants, suggesting that antigenic variation is controlled by a recombinational switch. The rearrangement seems to correlate only with antigenic variation and not the phase variation (or on-off switching) of flagella described for certain *C. jejuni* strains by Caldwell et al. (3). No rearrangements were found in DNA from motile and nonmotile variants of *C. jejuni* 81116 isolated either with motility medium or by rabbit passage as described by Caldwell et al. (3). Similarly, *C. jejuni* HC, which also undergoes a flagellar phase variation, failed to show any DNA rearrangements after animal passage. Unlike strains 81116 and HC, which display nonmotile variants at a high frequency when plated in motility plate medium, strain VC167 only rarely displays a totally nonflagellated phenotype, and once detected, such nonmotile variants are very unstable. Thus, phase and antigenic variation of flagella in *Campylobacter* spp. seem to be distinct events, although the potential for production of multiple serospecificities of flagellin has not been examined in strain 81116 or HC. The relationship between phase and antigenic variation in *Campylobacter* spp. needs to be better elucidated.

The observed rearrangements that occur during flagellar phase variation in strain VC167 clearly involve a programmed recombinational event, and an analysis of the way in which this rearrangement controls expression of surface antigens is under way. An understanding of the molecular nature of this event will require comparison of the 9.4-kb *Eco*RV fragment of phase 1 cells with the corresponding 8.4-kb *Eco*RV fragment of phase 2 cells. Sequence analysis of the DNA cloned in pGK109 will also contribute to our understanding of the rearrangement, as well as identify the nature of the observed homology between campylobacters and the enteric bacteria. Since DNA switches have been described that control expression of surface antigens of numerous pathogens (2), several models of control could be envisaged. These range from the classic invertible switch controlling phase variation of salmonellae (18) to the complex cassette system of antigenic variation of pilin in *Neis-*

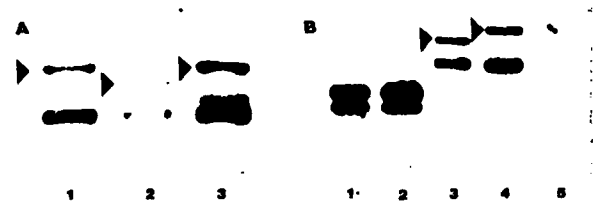

FIG. 4. Autoradiograms of Southern blots of *C. coli* VC167 DNAs probed with *S. typhimurium* LT2 (A) and pGK109 (B) (different photographic enlargements). (A) *Eco*RV digests of VC167 phase 1 (lane 1), VC167 phase 2 (lane 2), and VC167 phase 1, switched from phase 2 (lane 3). (B) Lane 1, *Hin*dIII-digested VC167 phase 2; lane 2, *Hin*dIII-digested VC167 phase 1; lane 3, *Eco*RV-digested VC167 phase 2; lane 4, *Eco*RV-digested VC167 phase 1; lane 5, *Hin*dIII-digested bacteriophage lambda. The arrowheads indicate bands that are involved in rearrangements. The molecular sizes of these bands are as follows: panel A, lane 1, 9.4 kb; panel A, lane 2, 8.4 kb; panel A, lane 3, 9.4 kb; panel B, lane 3, 8.4 kb; panel B, lane 4, 9.4 kb. Lane 2 in panel A contained less DNA than did the other lanes.

seria gonorrhoeae (12, 17). In addition, it has been demonstrated recently that antigenic and phase variation of another major surface protein of *N. gonorrhoeae*, the opacity protein, is regulated by a novel mechanism of translational control (16). The recent preliminary report of a method for generating mutations in cloned *Campylobacter* genes in *E. coli* and returning them to *Campylobacter* species (A. Labigne-Roussel, J. Harel, and L. S. Tompkins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, D89, p. 86) may form the basis for understanding the regulation of surface antigens of *Campylobacter* species at the genetic level.

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